REMARKS

Claims 1, 4, 5, 9-18 and 20-25 are pending in the present application.

Compared to the previous set of claims, please note that:

- previous claims 1, 4, 5, 9-18 and 20-25 are maintained without any amendment,
- previous claims 2, 3, 6-8 and 19 remain cancelled, and that
- previous claims 26-29 are now cancelled.

In the Final Office Action dated of June 28, 2006, all rejections have been withdrawn, except rejections under 35 U.S.C. 103(a).

Claims 1, 4, 5, 9-12 and 20-25 have been rejected under 35 U.S.C. 103(a) as being unpatentable over **Kalionis** in view of **Vona**.

Said Kalionis reference is the PCT international application WO 99/15892.

Said Vona reference is a scientific publication of January 2000 (Vona et al., American Journal of Pathology, vol. 156, No. 1, January 2000, pages 57-63).

Claims 13, 14, 16, 17 have been rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona, and further in view of **Bianchi** (US 5,614,628).

Claim 15 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona and **Fodor** (US 6,309,822).

Claim 18 has been rejected under 35 U.S.C. 103(a) as being unpatentable over Kalionis in view of Vona, and further in view of **Pinkel** (US 6,159,685).

Applicants respectfully traverse each of these rejections.

The Kalionis reference:

The Kalionis reference discloses a method by which a cell fraction that is enriched in trophoblast cells is isolated by filtration from a sample of peripheral blood of a pregnant mammal (*see e.g.,* claim 1 of the Kalionis reference).

According to this method, a sample of maternal blood is passed through a "selective filter", such as a filter having a pore-size of approximately 10 μm (*see e.g.,* page 7 line 13 of the Kalionis reference).

The cells that are retained on the filter are washed away from the filter, and re-suspended in a buffer (*see e.g.,* the paragraph bridging pages 7 and 8 of Kalionis).

The cell fraction that is thereby obtained is not a pure population of fetal cells (trophoblast cells), but is a mixture of various cell types, which is enriched in fetal cells (compared to the initial fetal cell concentration that is contained in the sample of maternal blood), but which also comprises maternal cells (*see e.g.,* page 4 lines 15-18 of the Kalionis reference). In the Kalionis reference, there is no step, wherein a fetal cell would be isolated. Hence, contrary to the claimed method of the present invention, the Kalionis reference does neither disclose nor suggest the isolation of fetal cells.

Once the cell fraction has been obtained, aliquots thereof are pipetted onto a solid support, such as a glass microscope microslide (*see e.g.,* page 8 lines 1-2 of the Kalionis reference).

In order to identify and quantify the trophoblasts within the various cells that are present on the solid support, immunostaining is performed using trophoblast-reactive antibodies (*see e.g.,* from page 8 line 4 to page 9 line 22).

The Kalionis reference discloses that genetic and/or biochemical information can be obtained by submitting the collected cells to *in situ* hybridization using:

- genetic probes for detecting trophoblast-specific mRNA, and/or
- genetic probes for identifying specific human chromosomes;

see e.g., page 10 lines 7-8, lines 17-18 and line 28 of the Kalionis reference.

In page 18, 4th paragraph, the Kalionis reference discloses that *in situ* hybridization can be carried out « following the detection of trophoblast cells using trophoblast-reactive antibodies », which would result in:

- filtering a sample of the maternal blood,
- collecting the cells that are retained on the filter,
- submitting the collected cells to immunostaining with trophoblast-reactive antibodies, to identify the trophoblast cells, and then
- submitting the immunostained cells to *in situ* hybridization.

It should be noted that the Kalionis reference is silent about which kind of *in situ* probes are intended to be used after said immunostaining step:

- the genetic probes that are specific to trophoblast mRNA?
- or the genetic probes that are "specific for human chromosomes"?
- or is it that both types of probes are intended to be used after said immunostaining step?

In the present US patent application, the claimed method schematically comprises:

- **filtering** a sample of the maternal blood (see step a) of claim 1 of the present application),

- **analyzing** the cells that are retained on the filter **to identify at least one cell that is presumed to be of fetal origin,** by identification of at least one immunological and/or cytological marker that is characteristic of trophoblastic and/or synciotrophoblastic cells, **and collecting a single cell that is presumed to be of fetal origin, or a collection of such single cells** (see **step b) of claim 1 of the present application**), and then
- **submitting one single cell that is presumed to be of fetal origin to nucleic acid amplification, both to demonstrate the fetal origin of the cell and to carry out the prenatal diagnosis** (see steps c)-e) of claim 1 of the present application), whereby the confirmation of the fetal origin and the prenatal diagnosis are both performed (by nucleic acid amplification) on the very same cell, more precisely on one single cell.

To more accurately follow the method that is currently claimed in the present US patent application, one would preferably presume that, in the Kalionis reference, both types of genetic probes are intended to be used after the immunostaining step.

Indeed, with this assumption in mind, the disclosed method of the Kalionis reference is said to comprise:

- submitting the collected cells to immunostaining with trophoblast-reactive antibodies, to identify the trophoblast cells, and then
- submitting the immunostained cells to a dual in situ hybridization, using:
    - probes that are specific for trophoblast mRNA, and
    - probes that are "specific for human chromosomes",

whereby the Kalionis reference is said to disclose a method, which would comprise a step that would resemble step b) of the claimed method, *i.e.*, a method, which would comprise an immuno-staining step, before nucleic acid analysis.

However, such an interpretation of the Kalionis reference is an undue interpretation of the actual content of the Kalionis reference, that has been made with foreknowledge of the claimed invention.

Indeed, **the immuno-staining step is, in the Kalionis reference, performed after a step, wherein the cells are collected, whereas in the claimed method of the present invention, the immuno-staining step is performed before the step of cell collection.**

A proper analysis of the actual content of the Kalionis disclosure would not omit the step of cell collection, and the fact that, in the Kalionis reference, the step of cell collection is performed before the immuno-staining step.

In other words, contrary to what is suggested in page 3 lines 4-43 of the outstanding Office Action, **the steps of the method of the Kalionis reference are not in the same order as the method of the present US application.**

The fact that the immuno-staining step is performed at a different time does of course further reflect the fact it does not have the same function. Indeed, the immuno-staining step of the Kalionis reference is not disclosed as being a step, such as step b) of the present invention, by which certain cells are detected as presumably being of fetal origin, to isolate these selected cells from the other cells.

On the contrary, in the Kalionis reference, all the cells that are retained on the filter are collected together in mixture, which means that the collected cells comprise fetal cells (trophoblast cells), but also comprise other circulating cells (notably, certain maternal cells); *see e.g.,* page 4 lines 15-18. The immuno-staining step is performed on the mixture of collected cells, without any isolation or any further enrichment in fetal cells.

The Kalionis reference consistently discloses that the immuno-staining step is performed after collection of the cells, to identify and quantify the fetal cells (see page 8 lines 4-5, 25 of the Kalionis reference).

In other words, assuming that the immuno-staining step that is disclosed in the Kalionis reference is a step that would resemble step b) of the claimed method of the present invention is not consistent with the content of the Kalionis reference.

Hence, contrary to what is written on page 3 lines 4-13 of the outstanding Office Action, the Kalionis reference does not disclose a method, which would generally comprise the steps of:

- submitting the collected cells to immunostaining with trophoblast-reactive antibodies, to identify the trophoblast cells, and then
- submitting the immunostained cells to a dual in situ hybridization, using:
    - probes that are specific for trophoblast mRNA, and
    - probes that are "specific for human chromosomes",

but discloses a method, which comprises:

- filtering a sample of the maternal blood,
- **collecting the cells that are retained on the filter, and then**
- submitting the collected cells to **immunostaining** with trophoblast-reactive antibodies, to identify the trophoblast cells, **and then**
- submitting the immunostained cells to ***in situ*** **hybridization**.

It is thus respectfully submitted that the analysis of Kalionis reference that is given on page 3 of the Office Action (see lines 4-13) has been made *ex post facto*, *i.e.*, that the Kalionis reference has been re-interpreted in the light of the content of the present application, having foreknowledge of what matter constitutes the claimed invention.

It is furthermore respectfully submitted <u>that the Kalionis reference does not disclose step b) of the claimed method</u>, *i.e.*, a step, which would comprise immuno-staining the cells before collecting an appropriate cell, *i.e.*, a cell the fetal origin of which would be presumed.

Moreover, to arrive at the claimed invention, the Kalionis reference has to be completely modified:

- you should not collect all the cells that have been retained on the filter, but you should collect one single cell (or a collection of selected single cells),
- you should not proceed to the immuno-staining step after collection of the cells, but before collection of the cells, and with the purpose of isolating a cell that is presumed of fetal origin,
- you should not use in situ hybridization, but you should use nucleic acid amplification, and
- this nucleic acid analysis should not be performed on a plurality of cells (namely, all the cells that have been retained on the filter), but on one single cell that has been previously characterized as being presumably of fetal origin.

In other words, starting from the Kalionis reference, you have to change everything except, maybe, the filtration step, to arrive at the claimed method.

Under such circumstances, the obviousness argumentation cannot be said to be persuasive and demonstrative.

Once a new idea has been formulated, it can often be shown theoretically how it might be arrived at, starting from something known, by a series of apparently easy steps.

However, the overall state of the art should be assessed without foreknowledge of the claimed invention, *i.e.*, before the applicant's contribution, and a "real-life" assessment of the prior art content should be made.

It is thus respectfully submitted that the obviousness rejection is not properly based, and that the argumentation presented in support thereof is based on an *ex post facto* analysis of the disclosure, that resulted in an erroneous interpretation thereof, and that the main document on which the rejection relies is so distantly related to the claimed invention, that it more likely demonstrates that the claimed invention is not obvious, than the contrary.

In the Kalionis method, there is no step wherein an immuno-labelled cell, being presumably of fetal origin, is individually collected (isolated) in order to assess the fetal origin of its DNA. And there is no step wherein such a cell (or such cells) would be isolated. Neither does it comprise a step wherein fetal cells would be isolated.

In the Kalionis reference, the immuno-staining step is considered to be sufficient to identify fetal cells, and nucleic acid analysis (FISH) is performed on a mixed fetal-maternal cell population to detect genetic abnormalities on the immuno-stained cells. In contrast, in the claimed invention, the immuno-staining step is considered to enable only a presumption of fetal origin, and the DNA analysis (DNA amplification) is performed on a single isolated cell, the fetal origin of which is presumed, to assess the fetal origin of this single cell and, if the fetal origin is demonstrated, to subsequently detect genetic abnormalities in the very same single cell (see step e) of claim 1).

As a matter of fact, the method of the Kalionis reference is suitable for the late stage of gestation, but not for the early stage. Indeed, as the Kalionis method is <u>not</u> performed on an isolated fetal cell (nor on isolated fetal cells), but on a mixed population, which is enriched in fetal cells (trophoblast cells), but which also comprises other cell types (including maternal cells), the Kalionis method is enabled for pregnant women, who are at the end of gestation, *e.g.,* in their 30-37 weeks of gestation, *i.e.*, <u>7½ months of gestation</u> and over (see Table 1 of the Kalionis reference, in page 21).

In contrast, the claimed method of the present application is enabled for pregnant women at any stage of pregnancy, and more particularly at the very early stages of pregnancy, *e.g.*, as early as 5 weeks of gestation (see page 7 lines 15 and 18 of the present application, as well as claim 20).

The method of the invention is precisely suited to the early stage of pregnancy. The claimed invention is precisely meant to address this problem.

Having a pre-natal diagnosis at 5 weeks of gestation has a real medical value for both the mother and the fetus. That is what the claimed invention enables. By comparison, the Kalionis reference, which describes a potential "chromosomic analysis" of the baby at 7½ months appears to have little value.

Also, the Kalionis reference teaches to wash out the fresh cells from the filter, to recover them by centrifugation and to proceed to cell analysis. This step carries a risk of loosing the rare trophoblastic cells. In contrast, the claimed method analyzes the cells that are retained (fixed) on the filter (step b) of the claimed method, whereby loss of target cells is avoided. It is respectfully submitted that the Kalionis method is not suitable for real-life clinical applications.

Conclusions on the Kalionis reference:

The Kalionis reference does neither disclose nor suggest a method as described in page 3 lines 4-13 of the outstanding Office Action.

The Kalionis reference does neither disclose nor suggest a method, which would comprise a step that would resemble step b) of the claimed method. The claimed method is only very distantly related to the Kalionis method.

As all rejections of the outstanding Office Action are 35 U.S.C. 103(a) rejections based on the Kalionis reference as the main prior art document, it is respectfully requested that all rejections should be reconsidered and withdrawn.

To further support this request for rejection withdrawal, the following additional arguments are made:

The Vona reference (Vona et al., 2000):

The Vona reference is a scientific publication, for which the inventor is a co-author. The inventor therefore has a good knowledge of the content of the Vona reference.

The Vona reference discloses the Isolation by Size of Epithelial Tumor cells (ISET). The ISET assay consists in filtering cells so as to recover epithelial tumor cells. The ISET assay is disclosed to be followed by various tumor cell detection assays, among which RT-PCR is cited: see page 61 of the Vona reference, left-hand column, last paragraph ("molecular analysis").

In the Vona reference, there is only one single assay comprising both ISET and RT-PCR. This (ISET + RT-PCR) assay is performed on an epithelial tumor cell line, namely the Hep3B cell line. In this assay, all the cells that are retained on the ISET filter are tumor cells, whereas, in the claimed method, the cells that are retained on the filter are a complex population comprising different cell types in mixture, namely fetal and maternal cells.

The Vona reference suggests to proceed to test whether ISET would be applicable to the filtration of trophoblast cells (see last paragraph in page 62, right-hand column). But, this reference does not suggest to apply (ISET + RT-PCR) or (ISET + PCR), or more precisely (ISET + immunological or cytological analysis + PCR), to trophoblast cells.

Applying (ISET + PCR), or more precisely (ISET + immunological or cytological analysis + PCR), to trophoblast cells is the result of a selection among the various trophoblast detection techniques that can be contemplated after an ISET assay.

As a matter of fact, **the Vona reference does not disclose a step that would resemble step b) of the claimed method,** *i.e.*, a step, which would comprise immunologically or cytologically analyzing the cells before collecting an appropriate cell therefrom, *i.e.*, a cell, the (fetal) origin of which would be presumed.

Hence, **neither the Kalionis reference nor the Vona reference discloses a step that would resemble step b) of the present invention**.

As a consequence, even if one would combine the Kalionis teaching to the one of the Vona reference, he/she would not arrive at the claimed method.

Furthermore, **the Vona reference does not disclose a method, wherein the amplification step would comprise the demonstration of two clinical features**. Indeed, the RT-PCR step that is disclosed in the Vona reference is intended for detecting only one clinical feature, i.e., the p53 mutation, whereas in the claimed method of the present invention, the amplification step is intended for both demonstrating the fetal origin of the collected cells and for carrying the prenatal diagnosis as such (see step e) of the claimed method).

It is thus respectfully submitted that even if one would combine the Vona reference to the Kalionis reference, he/she would arrive at a method, which would still differ from the claimed one.

Claim 1 of the present US patent application thus fulfills the non-obviousness requirement set out at 35 U.S.C. 103(a), in view of the Kalionis and Vona references.

Claims 4, 5, 9-18 and 20-25 are dependent of claim 1, and involve every feature of claim 1. As a consequence, these dependent claims also fulfill the non-obviousness requirement set out at 35 U.S.C. 103(a), in view of the Kalionis and Vona references.

As indicated in page 5 lines 8-10 of the present application, the claimed method allowing an enrichment in fetal cells by a factor of more than 6 million, whereas the population of fetal cells comprises two types of cells (notably, cytotrophoblasts and syncytiotrophoblasts, see *e.g.*, page 5 lines 13-16), *i.e.*, a cell population, which in addition to be at a very low cell concentration in the maternal blood (about one cell per mL), is a complex cell population.

It is respectfully requested that all rejections that are based on Kalionis in view of Vona should be reconsidered and withdrawn.

Secondary references:

In view of what precedes, it appears unnecessary to further comment on the secondary references, i.e., the Bianchi, Fodor and Pinkel references (which were all cited in combination with the Kalionis and Vona references). The arguments that were presented in our response to the previous Office Action are nevertheless maintained.

As a matter of fact, the secondary references do not overcome the deficiencies of the primary references, and they do not suggest or disclose the same invention.

Therefore, reconsideration and withdrawal of the rejection of claims 13-18 based in part on these secondary references are respectfully requested.

ENTRY OF AMENDMENTS

The amendments to the claims should be entered by the Examiner because the amendments are supported by the as-filed specification and drawings and do not add any new matter to the application. Additionally, the amendments should be entered since they comply

with requirements as to form, and place the application in condition for allowance. Further, the amendments do not raise new issues or require a further search since the amendments incorporate elements from dependent claims into independent claims and/or are supported by the as-filed specification. Finally, if the Examiner determines that the amendments do not place the application in condition for allowance, entry is respectfully requested since they certainly remove issues for appeal.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Thomas J. Siepmann, Reg. No. 57,374 at the telephone number of the undersigned below, to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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Respectfully submitted,

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